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Article

Paclobutrazol dosage to modify the visual appearance of *Lilium* CV. Litouwen

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Abstract

The doses of paclobutrazol that reduce the height of the flower stem or modify the visual appearance may be different for each cultivar. In this research, the effect of eight paclobutrazol concentrations on stem height, visual appearance, anthesis period, and biomass distribution of Asiatic *Lilium* cv. Litouwen was evaluated. Eighteen bulbs were immersed for 24 h in 5 L of the following solutions: 0 (control), 2.5, 5, 10, 20, 40, 100, 200 mg L^{-1} of paclobutrazol. After some time, three bulbs were planted in pots with 2.5 L of tezontle and randomly distributed inside a greenhouse. During the 2022 crop cycle, the moisture of the substrate was maintained with a Steiner solution. Results were processed with analysis of variance and Tukey's mean comparison tests (*p*# 0.05). The height of the flower stems was 61.5, 56.6, 43.4, 29.1, 18.4, and 14.1 cm with 0 or 2.5, 5 or 10, 20, 40, 100, and 200 mg L^{-1} of paclobutrazol, respectively. Leaf number and leaf area decreased, but stem diameter, greenness index, dry root biomass, flower buds, time to flowering and anthesis period increased from 40 mg L^{-1}. In *Lilium* 'Litouwen', doses of 40 or 100 mg L^{-1} of paclobutrazol are required to obtain compact plants with intense green foliage and striking flower buds, which would help their marketing as pot plants.

Keywords:

anthesis period, dry biomass, leaf area, number of leaves.

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Introduction

Within the genus *Lilium* (Liliaceae Juss) are the Asiatic and oriental hybrids of *Lilium longiflorum*, which are highly prized by consumers as cut flowers or potted plants (Bahr and Compton, 2004). The height of their flower stems, which range from 60, 84 or even 130 cm for *Lilium* 'Ercolano', 'Fangio' or 'Castello', could complicate their management as pot ornamentals (Auzaque-Rodríguez *et al*., 2009; Al-Ajlouni *et al*., 2017; Flores-Pérez *et al*., 2021). The use of growth regulators can help to solve this situation as they not only reduce the length of the stems but can also increase the number of flower buds per plant or improve the intensity of color in the petals and foliage (Davies, 2010; Rademacher, 2015).

For example, in *Lilium longiflorum*, foliar application of 150 mg L^{-1} of gibberellic acid (GA_3) + 75 mg L^{-1} of 6-benzylaminopurine increases the number of buds per flower stem from 4 to 5.5 (Emami *et al*., 2011). In *Euphorbia pulcherrima*, height increases from 20.3 to 25.8 cm by spraying 4, 6, 8 or 10 mg L^{-1} of GA_3 (Alia-Tejacal *et al*., 2011). Conversely, growth retardants such as paclobutrazol inhibit the biosynthesis of active gibberellins (including GA_3) by blocking the kaurene oxidase enzyme and preventing the oxidation of kaurene acid into ent-kaurenoic acid (Taiz and Zeiger, 2010).

The decrease in GA_3 reduces the length of the internodes and modifies the visual appearance by forming plants with more compact canopies (Rademacher, 2016). Francescangeli *et al*. (2007) mention that, flowering stems of hybrid *Lilium* cv. Ercolano reduce their height by 29, 34 and 46% by applying 50, 100 or 150 mg L^{-1} of PBZ (control, 64.4 cm), without affecting the length or number of flower buds. Rios *et al*. (2022) mention that the height of *Lilium* 'Armandale' decreased from 59.1 (control) to 51.6 or 38.9 cm due to the application of 50 or 200 mg L^{-1} of PBZ, but in *Lilium* 'Tresor' this effect is not so noticeable, in both cultivars the number of flower buds increases from 2.7 to 4.2 with 200 mg L^{-1} of PBZ. Torres-Pio *et al*. (2021) mention that in *Lilium* 'Arcachon', the height of the flower stems decreases 4.1 or 5.3 times when applying 25 or [50, 100, and 200] mg L^{-1} of PBZ (control, 85.3 cm), while the number of buds remains at 5.9 and their fresh weight increases due to PBZ.

These changes give *Lilium* 'Arcachon' a striking visual appearance with the possibility of being marketed as potted plants. The application of growth regulators has been a common practice to modify the visual appearance of ornamentals. In *Lilium*, for example, PBZ concentrations ranging from 25 to 200 mg L^{-1} have been tested to shorten the length of flower stems.

However, it is possible that lower doses could provide the physiological response that is required, which would allow product to be economized and reduce possible residual effects. Therefore, this research aimed to evaluate the effect of eight paclobutrazol concentrations on stem height, visual appearance, anthesis period and biomass distribution of Asiatic *Lilium* cv. Litouwen.

Materials and methods

The bulbs of Asiatic *Lilium* cv. Litouwen were purchased from the company Flores de Bulbos Importados SA de CV, Villa Guerrero, State of Mexico, Mexico, and transported to the laboratory where the excess peat moss was removed; they were washed with distilled water, separated into eight groups of 18 bulbs each and immersed in 5 L of the following solutions or treatments: 0 (control, distilled water), 2.5, 5, 10, 20, 40, 100, 200 mg L^{-1} of PBZ for 24 h.

After some time, three bulbs were planted in a plastic pot with 2.5 L of tezontle (granulometry # 5 mm), thus forming six experimental units or repetitions per treatment. The experimental units were randomly distributed and remained, in 2022, inside a greenhouse with a plastic cover.

The humidity of the substrate was recorded with a tensiometer from Irrometer® Co, USA, and was kept between 80 and 100% by applying a nutrient solution with pH of 6 and electrical conductivity of 2 dS m^{-1} (Steiner, 1961). To avoid fertilizer precipitation, they were irrigated with acidulated water (pH, 6) once a week, while the plants were monitored to prevent the incidence of pests or diseases. The following was evaluated:

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Flower stem height

It was recorded weekly with a Truper® tape measure, accuracy of 0.01 cm, from their base to the meristematic apex.

Stem diameter, number of leaves, leaf area, and greenness index

In the middle part of each plant, the diameter of the stem was recorded with a Truper® digital vernier with accuracy of 0.01 mm; the number of leaves was obtained by direct counting, the leaf area with a Li-Cor model Li-3000A leaf area integrator, and the greenness index in the leaves of the middle part with a Hansatech® model CL-01 portable SPAD meter.

Visual appearance of flower stems and roots

For the visual appearance, images were constructed from photographs taken with a Nikon® D3500 digital camera and processed with Adobe Photoshop® CS6.

Number, length and fresh weight of flower buds, time to flowering and anthesis period

In each stem, the number of flower buds was counted directly; the length of each one was obtained with a Truper® digital vernier with accuracy of 0.01 mm; the fresh weight was recorded with an Ohaus Adventurer® model AR3130 digital balance with accuracy of 0.01 g; the time to flowering was obtained by counting the number of days elapsed from planting to the opening of the first flower bud, and the period of anthesis by counting the days that elapsed from flowering to senescence.

Dry biomass of roots, bulbs, stems, leaves, and flower buds

Three flower stems per treatment were selected to be fractionated into roots, bulbs, stems, leaves, and flower buds. Each one had its fresh biomass recorded, they were placed in paper bags, and dried at 80 °C for 24 h in a Reavel® model REA-II-2240 oven; the dry biomass was obtained with the same balance.

Statistical analysis

Data were processed with descriptive statistics, one-factor analysis of variance, and mean comparison tests (Tukey, $p \leq 0.05$) with the SAS® 9.0 software for Windows.

Results and discussion

Flower stem height

In Asiatic *Lilium* cv. Litouwen, the application of PBZ decreased the height of the flower stems from day 28 (beginning of the vegetative phase) and it became more evident at flowering (84 to 91 d). In this phase, stems with 0 or 2.5 mg L^{-1} were 61.5 cm tall, in those with 5 or 10 mg L^{-1} it decreased to 55.6 cm, and for concentrations of 20, 40, 100, and 200 mg L^{-1} of PBZ, the height of the flower stems was 43.4, 29.1, 18.4, and 14.1 cm, respectively (Figure 1).

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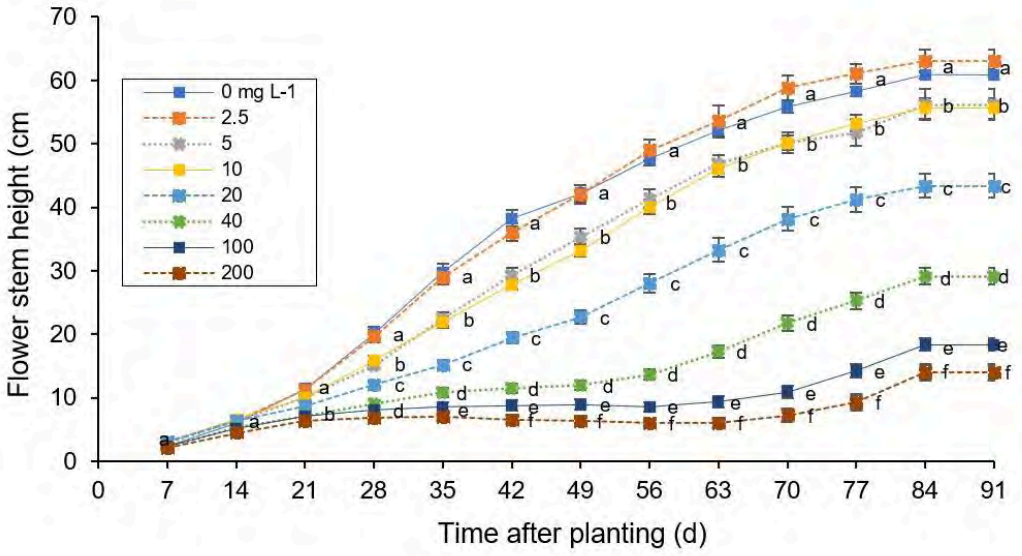
Figure 1. Height of the flower stems of Asiatic *Lilium* cv. Litouwen grown with eight concentrations of paclobutrazol. Means with different letters, at each evaluation time, indicate significant differences (Tukey, $p \leq 0.05$). Each data represents the average of six experimental units (eighteen flower stems) ± standard error.



Results such as those shown by Francescangeli *et al*. (2007); Currey and López (2010); Latimer and Freeborn (2011), mention that *Lilium* cv. Ercolano, Royal Respect, Nellie White, and *Lilium lancifolium* reduce between 23 and 59% the height of their flower stems, which is 63 cm, when applying 20 to 150 mg L^{-1} of PBZ. In *Lilium* cv. Litouwen, its height decreased between 9.6 and 77.1% compared to the control (61 cm).

Stem diameter, number of leaves, leaf area, greenness index

Stem diameter was similar (6.92 ±0.13 mm) in treatments with 0, 2.5, 5, 10 or 20 mg L^{-1} of PBZ, but increased by 51% in those with 100 or 200 mg L^{-1}. The number of leaves and leaf area decreased from 77 to 32.3 and from 658.55 to 227.93 cm^2 from 0 to 200 mg L^{-1}, while the greenness index increased from 25.21 to 75.77 USPAD with the same doses of PBZ (Table 1).

Table 1. Variables evaluated during the flowering phase (84 days after planting) in Asiatic *Lilium* cv. Litouwen grown with eight concentrations of paclobutrazol (PBZ).

PBZ concentrations (mg L^{-1})	Stem diameter (mm)	No. of leaves	Leaf area (cm^2)	Greenness index (USPAD)
0	6.5 b[z]	77 a	658.55 a	25.21 e
2.5	7 b	74.7 a	661.04 a	41.1 d
5	6.6 b	63 b	534.54 b	42.38 d
10	7.3 b	60.3 b	536.62 b	48.59 d
20	7.2 b	52.3 c	460.98 b	59.42 c
40	8.7 ab	42 d	363.44 c	62.06 bc
100	10.9 a	34.3 e	257.3 d	69.55 ab
200	10 a	32.3 e	227.93 d	75.77 a
HSD	2.6	5.8	77.08	9.74

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PBZ concentrations (mg L^{-1})	Stem diameter (mm)	No. of leaves	Leaf area (cm^2)	Greenness index (USPAD)
CV (%)	11.6	3.8	5.89	13.16

^{z} = means with different letters, in each column, indicate significant differences (Tukey, $p \leq 0.05$). HSD= honest significant difference; CV= coefficient of variation. Each value represents the average of three repetitions.

In Asiatic *Lilium* cv. Litouwen, the larger diameter of the stem due to the application of 40, 100 or 200 mg L^{-1} of PBZ could be related to an increase in the cortex and medulla or to the greater number of vascular bundles, medulla cells and vessel elements, as mentioned by Tsegaw *et al*. (2005); Torres-Pio *et al*. (2021) for *Solanum tuberosum* and *Lilium* cv. Arcachon, respectively.

In treatments with 100 or 200 mg L^{-1}, the decrease in the number of leaves coincides with the reduction in leaf area, but contrasts with the greenness index. If the visual aspect, which can be seen in Figure 2, the number or length of flower buds (Table 2) are referred to, the previous answer would indicate that the photosynthetic efficiency per unit area increased in treatments with 100 or 200 mg L^{-1} of PBZ, as occurs in plants that are subjected to some type of stress (Taiz and Zeiger, 2010).

Figure 2 . Visual appearance of Asiatic *Lilium* cv. Litouwen grown with eight concentrations of paclobutrazol.

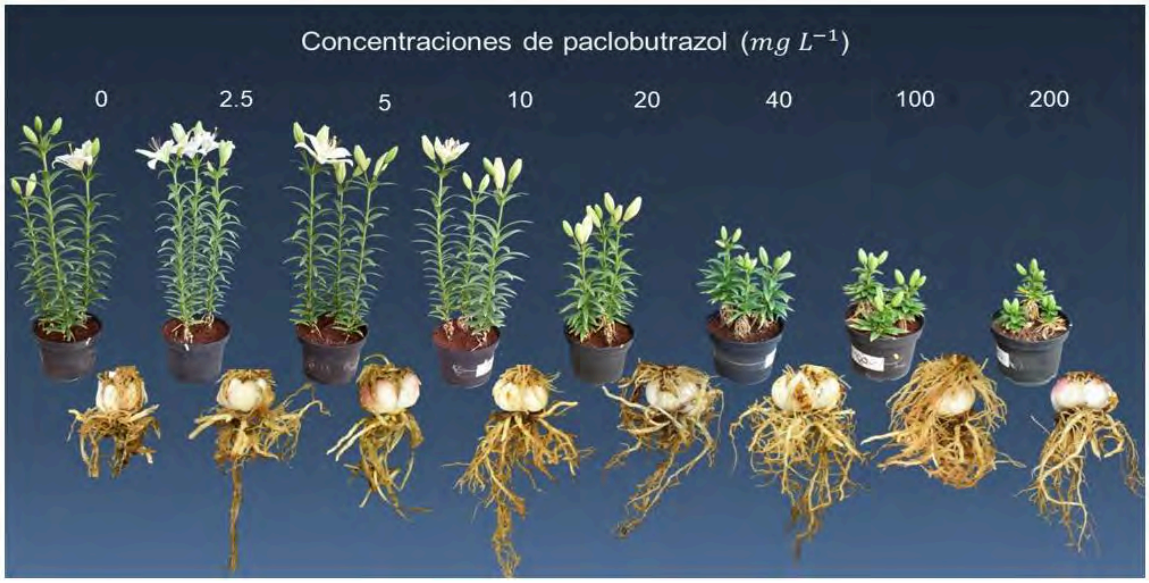

Table 2. Variables evaluated in the flowering phase of Asiatic *Lilium* cv. Litouwen grown with eight concentrations of paclobutrazol (PBZ).

PBZ concentrations (mg L^{-1})	Number	Flower buds Length (mm)	Fresh weight (g)	Time to flowering (d)	Anthesis period (d)
0	2.67 a^z	78.44 a	12.21c	87.5 d	7.17 f
2.5	2.33 a	81.19 a	12.24 c	93.3 cd	7.83 de
5	2.67 a	75.51 a	11.98 c	93.14 cd	8.17 de
10	2.67 a	74.44 a	14.5 bc	94.5 bc	10.67 cd
20	2.67 a	77.72 a	12.96 c	94.5 bc	12.67 c
40	2.33 a	61.7 b	20.34 ab	100.18 ab	13.17 bc
100	3.33 a	42.58 c	20.79 ab	103.6 a	16.33 ab
200	3.33 a	43.08 c	22.46 a	105 a	17.5 a

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PBZ concentrations (mg L^{-1})	Number	Flower buds Length (mm)	Fresh weight (g)	Time to flowering (d)	Anthesis period (d)
HSD	1.63	10.5	7.36	6.55	3.38
CV (%)	20.99	7.67	16.35	4.3	15.71

^{z} = means with different letters, in each column, indicate significant differences (Tukey, $p \leq 0.05$). HSD= honest significant difference; CV= coefficient of variation. Each value represents the average of three repetitions.

Visual appearance of flower stems and bulbs

The flowering stems of Asiatic *Lilium* cv. Litouwen treated with 0, 2.5, 5 or 10 mg L^{-1} of PBZ had similar visual appearance, intense green foliage, flower buds with good appearance and striking. The bulbs were turgid, with no damage or visible symptoms of disease, with similar root density, except in those treated with 10 mg L^{-1}, which had more numerous and longer roots.

From 20 mg L^{-1} of PBZ, the flower stems were more compact, reduced in height, but with no effect on the number of flower buds, these changes gave them a striking visual appearance. The bulbs maintained their good appearance, with no signs of damage, but the root density increased (Figure 2).

In *Lilium* cv. Ercolano, Royal Respect, Nellie White, Arcachon, Armandale or Tresor, application of PBZ in concentrations ranging from 20 to 200 mg L^{-1} reduces flower stem height, increases color intensity and root density compared to the control (0 mg L^{-1} of PBZ) (Francescangeli *et al*., 2007; Currey and López, 2010; Torres-Pio *et al*., 2021; Rios *et al*., 2022).

A similar response occurred in this research with Asiatic *Lilium* cv. Litouwen since with concentrations of 2.5, 5 or 10, no visual changes were perceived, but with 20, 40, 100 or 200 mg L^{-1} of PBZ, the flower stems were more compact and reduced in size, which facilitates their handling as potted plants and expands the possibilities for their commercialization with a more diverse public. Regarding the physiology of the crop, the increase in root density could improve the absorption of water and essential elements for the hydration or nutrition of the leaves or flower buds.

Number, length and fresh weight of flower buds, time to flowering and anthesis period

Regardless of the treatments, the number of flower buds was 2.75 ±0.14, while their length decreased from 78.44 (with 0, 2.5, 5, 10 or 20 mg L^{-1} of PBZ) to 42.58 or 43.08 mm with 100 or 200 mg L^{-1}. The fresh weight of flower buds, the days to flowering and the anthesis period increased by the application of 40, 100 or 200 mg L^{-1} of paclobutrazol (Table 2).

Findings such as those presented by Ranwala *et al*. (2002); Currey and Lopez (2010); Torres-Pio *et al*. (2021) mention that, in *Lilium* cv. Ercolano, Nellie White or Arcachon, the application of PBZ in concentrations ranging from 30 to 200 mg L^{-1} reduces stem height and keeps the number of flower buds (5.7 on average) and the days to flowering (72.5 in 'Ercolano'; 111 in 'Nellie White') unchanged. In Asiatic *Lilium* cv. Litouwen there were only 2.75 buds per flower stem as temperatures inside the greenhouse fluctuated between 30 and 43 °C from 10:30 am. to 6:00 pm. during their cultivation, which led to absorption of the flower buds.

Research by Runkle (2018) mentions that the Asiatic *Lilium* crop performs well with daytime temperatures of 21 to 27 °C, but when these exceed 29.4 °C, flower bud abortion occurs (Evans and Beck, 2007). The fresh weight coincided with that reported by Torres-Pio *et al*. (2021) for *Lilium* 'Arcachon'. The time to flowering is delayed and the anthesis period is doubled by the application of 40, 100 or 200 mg L^{-1} of PBZ.

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Dry biomass of roots, bulbs, stems, leaves, and flower buds

The dry biomass of roots, bulbs or flower buds increased 2, 2.2 or 1.6 times by the application of PBZ at concentrations ≥ 20 mg L^{-1}. In contrast, in stems and leaves, dry biomass decreased three and two times with respect to the control, the values of which were 2.85 and 2.36 g, respectively (Table 3).

Table 3. Distribution of dry biomass in Asiatic *Lilium* cv. Litouwen grown with eight concentrations of paclobutrazol (PBZ).

PBZ concentrations (mg L^{-1})	Dry biomass (g)				Flower buds
	Roots	Bulbs	Stem	Leaves	
0	0.61 c[z]	1.14 d	2.85 a	2.36 ab	1.31 bc
2.5	0.6 c	1.28 cd	2.6 ab	2.43 a	1.24 c
5	0.71 c	0.98 d	2.68 ab	1.85 bc	1.21 c
10	1.01 b	1.33 cd	2.34 ab	1.8 c	1.24 c
20	1.15 ab	1.51 cd	1.88 bc	1.8 c	1.28 bc
40	1.12 ab	1.89 bc	1.32 cd	1.23 d	1.87 ab
100	1.25 ab	2.37 b	1.03 d	1.12 d	2.23 a
200	1.33 a	3.35 a	0.7 d	0.73 d	2.29 a
HSD	0.26	0.62	0.96	0.52	0.63
CV (%)	9.63	12.69	21.02	11.06	14.05

[z] = means with different letters, in each column, indicate significant differences (Tukey, $p \leq 0.05$). HSD= honest significant difference; CV= coefficient of variation. Each value represents the average of three repetitions.

Dry root biomass could be related to the absorption of water and essential elements that are required for crop growth and flowering since a root system with abundant and long roots has a greater capacity for exploration compared to scarce and short roots (Taiz and Zeiger, 2010). In this case, the dry root biomass of Asiatic *Lilium* cv. Litouwen increased by the application of 20 to 200 mg L^{-1} of PBZ, which was able to improve the absorption of essential elements to increase the biomass of bulbs or flower buds.

This combination maintained the good appearance of the flower stems (Figure 2). On the other hand, the decrease in dry biomass in stems would imply a reduction in the vascular system, particularly phloem for the translocation of photoassimilates, while a lower dry biomass in the leaves would be associated with a reduction in the photosynthetically active area, which would negatively affect the visual appearance of the flowering stems. Nevertheless, this was not the case, suggesting an increase in translocation capacity or photosynthetic efficiency per unit area (Taiz and Zeiger, 2010).

Conclusions

Stem height decreased with concentrations ranging from 5 to 200 mg L^{-1} of PBZ, higher doses produced more compact plants. With 5 or 10 and 100 or 200 mg L^{-1}, the reduction had the same intensity. The number of leaves and leaf area decrease from 5 mg L^{-1} of PBZ, but the stem diameter and greenness index increase from 40 mg L^{-1}. High values of the greenness index give the foliage a greener and more striking appearance.

The number of buds was not affected by PBZ; however, their length decreased, while the time to flowering and the anthesis period lengthened and maintained the good appearance of Asiatic *Lilium* cv. Litouwen for longer. To decrease the height of the stems or modify the visual appearance of this cultivar, it is required that, before planting, the bulbs be immersed for 24 h in 20, 40 or 100 mg L^{-1} of paclobutrazol.

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With concentrations less than 20, the response would be similar to not adding any doses and concentrations greater than 100 mg L^{-1} would be product waste. With this management, the risks of possible residual effects are reduced, in addition to obtaining plants that are more easily manageable for marketing as pot ornamentals.

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anthesis period
dry biomass
leaf area
number of leaves

Counts

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